

## MYDRIASIS AND THE INCREASE IN OUTFLOW OF AQUEOUS HUMOR FROM THE RABBIT EYE AFTER CERVICAL GANGLIONECTOMY IN RELATION TO THE RELEASE OF NOREPINEPHRINE FROM THE IRIS\*

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**Abstract**—Tritiated norepinephrine administered intravenously in small single doses to albino rabbits is taken up and retained by the iris, then lost into the anterior chamber. In the early hours after cervical ganglionectomy the iris retention phenomenon exhibits a progressive decline while the release of the radioactively labeled norepinephrine is enhanced. The time course of these events could well explain the transient mydriasis and increase in outflow facility noted in the eyes of these animals after cervical ganglionectomy.

IT HAS been shown that the transient mydriasis and increase in facility of outflow of aqueous humor, which occur 20 hr after unilateral cervical ganglionectomy in the albino rabbit, coincide with the beginning disappearance of norepinephrine from the iris and ciliary body.<sup>1</sup> These observations support the hypothesis that norepinephrine is liberated from degenerating nerve endings into the aqueous humor and acts upon the dilator muscle of the iris<sup>2</sup> and outflow channels of the rabbit eye,<sup>3</sup> phenomena similar to the degeneration contraction of denervated nictitating membrane.<sup>4</sup> The fact that the outflow facility of the rabbit eye can respond to exogenous norepinephrine,<sup>4, 5</sup> and in particular that it increases maximally with injection of small amounts of norepinephrine (as little as 20 m $\mu$ g) close to structures concerned with aqueous outflow,<sup>6</sup> further affirms the plausibility of this hypothesis. No data have been presented, however, to demonstrate the actual release of norepinephrine from ocular tissues into the aqueous humor.

The present study presents evidence which indicates that after ganglionectomy, norepinephrine is released from ocular structures into the aqueous humor in a manner consonant with the physiologic events observed after denervation.

### METHODS

Chromatographically pure *d,l*-norepinephrine-7-<sup>3</sup>H·HCl (specific activity 7-14 mc/ $\mu$ mole) was obtained from the New England Nuclear Co., Boston. For use, a solution

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containing 10  $\mu\text{g}$  of the base/ml of 2% sodium metabisulfite was prepared and acidified with 0.2 N hydrochloric acid.

New Zealand male albino rabbits weighing 2.0 kg were maintained on a diet of pellets and water *ad libitum*. They were subjected to right cervical ganglionectomy under thiopental anesthesia.<sup>2</sup>

At intervals after ganglionectomy, each rabbit received 5  $\mu\text{g}$  of  $^3\text{H}$ -norepinephrine/kg (170  $\mu\text{C/kg}$ ) via the left marginal ear vein administered in a volume of 2.0 ml of normal saline washed in with an additional 2–3 ml. Two hours later the animals were killed with an overdose of 1% thiopental sodium given intravenously. The eyes were immediately proptosed. Samples of posterior chamber and anterior chamber fluid were withdrawn, and the iris and ciliary processes were removed from each eye. Sampling of right and left eyes was alternately performed to randomize any differences that might occur as a result of the 3-min delay before the processing of the second eye. Hamilton precision-ground 50- and 100- $\mu\text{l}$  syringes with permanently mounted 27-gauge needles were used throughout for obtaining aqueous humor.

Twenty  $\mu\text{l}$  of posterior chamber and 80  $\mu\text{l}$  of anterior chamber fluids were placed in scintillation counting vials containing 10 ml phosphor.<sup>7</sup> The irises were scraped clean, rinsed with iced distilled water, blotted, and weighed. Then each iris was minced and homogenized in 3.0 ml of 0.4 M perchloric acid and placed in preweighed centrifuge tubes so that the dry weight of the sediments could be obtained; 0.5 ml of distilled water used to rinse the grinding tubes was added and the material centrifuged for 5 min at 13,000 rpm at 0°. The extract volumes were measured and 0.2 ml placed in counting vials containing 10 ml Bray's phosphor. Radioactivity was measured in a Packard Tri-Carb liquid scintillation counter. Quenching was determined by the method of internal standards, with tritiated water. The following average quenching values were found for 20  $\mu\text{l}$  of posterior aqueous, 80  $\mu\text{l}$  of anterior aqueous, and 0.2 ml of iris extract respectively: 3, 12 and 46 per cent.

The results obtained were then plotted as a ratio of tritium, i.e. RE/LE  $\pm$  S.E.M. for each group as a function of the time after ganglionectomy. A minimum of six animals was used for each time interval after ganglionectomy.

To determine whether the labeled norepinephrine had been catabolized during the 2-hr interval between intravenous administration of the drug and removal of the aqueous and tissue samples, extracts of iris were assayed for total tritium and were then passed over alumina columns.<sup>8</sup> The  $^3\text{H}$ -norepinephrine of the hydrochloric acid eluates was measured and the results corrected for an average recovery of 80 per cent. Recovery of known amounts of  $^3\text{H}$ -norepinephrine was determined in duplicate during each analysis. Iris and ciliary body preparations removed from the right eyes at 14–17, 18, 19, 20–24 hr, and 36–50 hr after ganglionectomy were pooled and constituted five separate groups. Left eyes, used as controls, were taken from the 20–24-hour group.

## RESULTS

Neither the normally innervated nor the denervated irises showed significant catabolism of  $^3\text{H}$ -norepinephrine within the 2-hr interval between administration of the drug and tissue removal (Table 1). Even in a few experiments done at the longer time interval of 3.5 hr, the evidence for accumulation of metabolites is small. The results indicate that most of the label is still present as norepinephrine.

The comparative uptakes for right and left irises are indicated in Fig. 1. It is clear that no significant difference in uptake between the right denervated and normally innervated left eyes occurs until 18 hr, at which time a significant, progressive decrease in retention on the denervated side begins. The normally innervated left side had a

TABLE 1. PERCENTAGE OF D,L-7-<sup>3</sup>H-NOREPINEPHRINE·HCL RECOVERED FROM IRIS EXTRACTS

Source	(n)	Radioactivity associated with norepinephrine (%)	Time after ganglionectomy (hr)
Tissues removed 2 hr after i.v. administration of d,l-7- <sup>3</sup> H-norepinephrine. HCl			
Right iris	(8)	98	14-17
Right iris	(6)	72	18
Right iris	(10)	93	19
Right iris	(17)	85	20-24
Left iris	(17)	100	20-24
Tissues removed 3-5 hr after i.v. administration of d,l-7- <sup>3</sup> H-norepinephrine. HCl			
Right iris	(3)	59	18
Right iris	(5)	77	20-22
Left iris	(5)	100	20-22

consistent uptake and retention throughout. Expressed in counts/min  $\times 10^3$ /g wet weight, this was  $353.7 \pm 17.8$  ( $n = 46$ ). This amount of radioactivity corresponds to approximately  $0.04 \mu\text{g}$  of d,l-norepinephrine-7-<sup>3</sup>H·HCl retained per gram of iris and ciliary body.

The appearance of tritium in the aqueous of the anterior chamber is shown in Fig. 2. Within 2-3 hr of the time when uptake and retention of norepinephrine by the denervated iris are declining rapidly, the tritium appears in greatest concentration in the aqueous of the anterior chamber. Shortly after 24 hr, little or no retention by the iris occurs, and consequently there is no release of norepinephrine into the aqueous during this period (36-50 hr).

The appearance of labeled norepinephrine in the aqueous of the posterior chamber is shown in Fig. 3. No significant differences in the amounts of norepinephrine appearing in the aqueous humor of the posterior chamber were found at any time interval when normal and denervated sides were compared. An impression is derived, however, that a trend toward an increased amount of norepinephrine in the denervated posterior chamber occurs at the 20-24-hr interval after ganglionectomy (see Discussion below).

## DISCUSSION

Twenty hours after ganglionectomy, a progressive decline in the degree of retention of intravenously administered norepinephrine by the denervated iris and ciliary body occurs. At 36-50 hr, retention is negligible. The amount of labeled material found in the anterior chamber at this time is small because during the interval preceding this period the iris showed only little uptake and retention. The appearance of significant

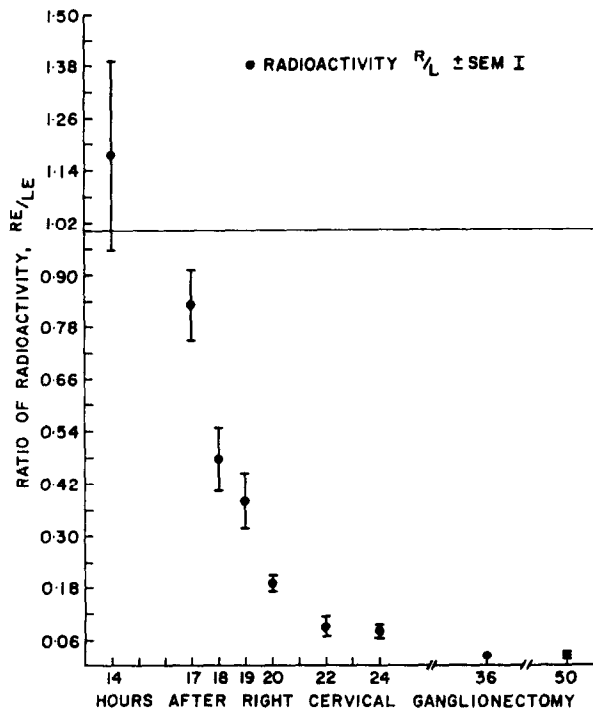


FIG. 1. The retention of tritiated norepinephrine by the iris and ciliary processes of rabbits after unilateral cervical ganglionectomy.  $^3\text{H}$ -Norepinephrine,  $5 \mu\text{g}/\text{kg}$  ( $170 \text{ m}\mu\text{c}/\text{kg}$ ), was injected i.v., and 2 hr later tissues were removed at the intervals after ganglionectomy indicated on the abscissa. Results were plotted on the ordinate as a ratio of counts/min of tritium, RE/LE (denervated/normally innervated iris). Each experimental point represents the mean  $\pm$  S.E.M. of at least six animals.

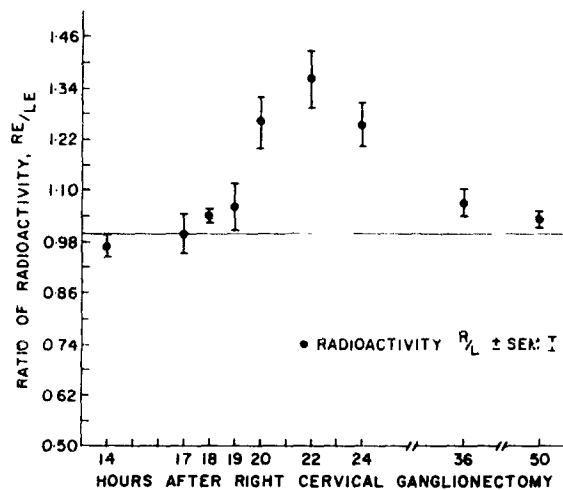


FIG. 2. The appearance of tritiated norepinephrine in the anterior chamber of rabbits after unilateral cervical ganglionectomy. Anterior chamber aqueous humor samples were handled as were the irises (see Methods and Fig. 1).

amounts of label in the right denervated anterior chamber during the 20-24-hr period, however, must be the result of prior uptake and retention by the iris and subsequent release into the aqueous humor.

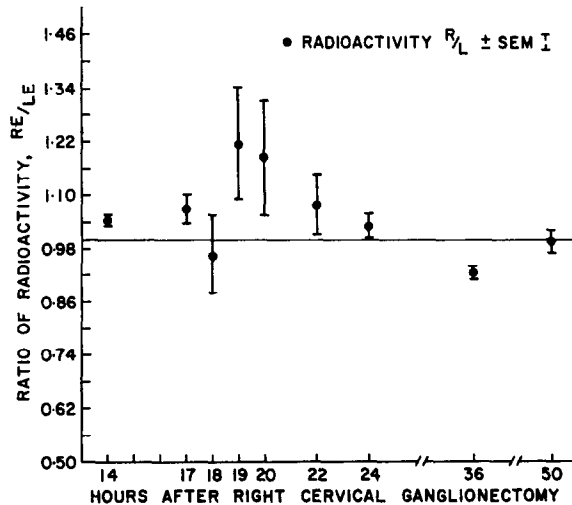


FIG. 3. The appearance of tritiated norepinephrine in the posterior chamber of rabbits after unilateral cervical ganglionectomy. Posterior chamber aqueous humor samples were handled as were the irises (see Methods and Fig. 1).

Now the greatest portion of the label in the aqueous humor must be associated with norepinephrine, because the present experiments indicate that little catabolism of iridial norepinephrine (Table 1) takes place exactly during those hours when the label is at its peak concentration in the aqueous. Positive identification of the aqueous label could be accomplished only by pooling a large number of individual aqueous samples. However, the only way that the aqueous label could come to exist in metabolite form would be if the aqueous itself had catechol *o*-methyl transferase or monamine oxidase activity. These enzymes have not been found in the aqueous.<sup>9</sup> For these reasons it is assumed that the radioactive material in the aqueous is not substantially different from that in the iris, although positive identification of the former has not been demonstrated.

The release of norepinephrine into the anterior chamber certainly could explain the transient mydriasis and increased outflow facility which occur after cervical ganglionectomy in the albino rabbit.<sup>1</sup> These parameters of ganglionectomy reach their maxima at the 20-24-hr period and so coincide with the release phenomenon demonstrated in this study. The response of the pupil and outflow facility to intra-cameral norepinephrine has already been demonstrated,<sup>6</sup> and the dependence of the response on alpha receptors has been established by alpha adrenergic blockade performed either via the anterior chamber<sup>3</sup> or via systemic administration.<sup>2, 6</sup>

Therefore the hypothesis that after ganglionectomy the anterior chamber aqueous mediates the events occurring in the pupil and outflow channels gains additional support. The explanation for transient mydriasis seems clear. It is possible, however, that adrenergic nerve endings in the chamber angle or in the intrascleral outflow

channels could mediate the increase in outflow facility. Adrenergic blockade with intracameral phenoxybenzamine<sup>3</sup> could have been established via innervated structures in the angle rather than via adrenergic receptors in the iris. Evidence for adrenergic innervation of the rabbit chamber angle is not strong,<sup>10, 11</sup> although the guinea pig angle is richly innervated.<sup>11</sup> Further experiments are in progress to test the participation of these nerves in the effect of ganglionectomy on outflow facility.

The question may be raised as to why a small amount of norepinephrine appeared in the denervated posterior chamber. If this were the result of increased blood flow to the denervated side, an expected increase in posterior chamber norepinephrine should be present at each time interval studied. This was not observed. Furthermore, if considerations of enhanced diffusion, based upon increased aqueous inflow rather than on increased blood flow, are important, then one must postulate a plasma posterior chamber gradient for norepinephrine. One hour after intravenous administration of labeled norepinephrine, however, essentially zero concentrations are found in the plasma. The most likely explanation of the observed trend toward an increase in posterior chamber norepinephrine at 20 hr appears to be that the iris takes up norepinephrine from the aqueous humor, viz. pupillary response, and then some of the drug is carried by the radial iris vessels into the marginal vein<sup>12</sup> whence it diffuses into the posterior chamber. A second contribution factor may be the presence of small numbers of adrenergic nerves in the stroma of the ciliary processes<sup>10</sup> which take up norepinephrine but then release it as do the iris nerves at the 20-hr interval after ganglionectomy.

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